The Effect of Steviol and Hyperthermia on Murine Mammary Adenocarcinoma Cells

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Background:

Studies have shown how chemotherapy plays a significant role in inducing apoptosis in neoplastic cells. However, most tumors become resistant, with many chemotherapy drugs failing to completely activate apoptotic pathways while differentiating between normal and cancerous cells.[1] Thus, there is a need of a additional therapeutic approaches that can recognize and effectively target and kill tumor cells, while minimizing effect to normal cells and tissue. Phytochemicals extracted from plants have a wide range of pharmacological applications an may be developed into important cancer therapies.[2]

Stevioside (triglucosylated steviol) is a diterpenoid glycoside isolated from **S. rebaudiana Bertoni** leaf (Fig. 1). It is hydrolyzed to steviol and glucose in the gastrointestinal tract by bacteria.[3] It has been reported as being anti-hyperglycemia,[4] anti-hypertensive,[5] anti-inflammatory,[6] immunomodulatory,[7] anti-tumor,[8] and anti-cancerous.[9] Recently, it was reported that steviol induced apoptosis in MCF-7 cells via DNA damage by mediating G2/M-phase arrest and reducing the level of reactive oxygen species (ROS). Rapid decrease in ROS after exposure to steviol shows the contribution of ROS in steviol induced apoptosis.[10]

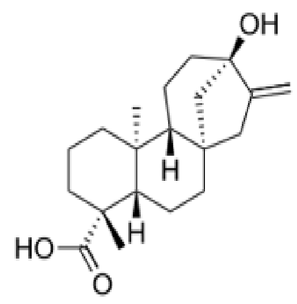


Fig. 1: Structure of Steviol ($C_{20}H_{30}O_3$

Hyperthermia involves increasing the body temperature above normal (above 37^0C). Studies have shown that high body temperature may damage or kill cancer cells, while minimizing damage to normal cells and tissues.[11] It enhances the cytotoxicity of many different antineoplastic drugs.[12]

Proposed Hypothesis:

Steviol has been reported to mediate G2/M phase arrest in response to DNA damage thus preventing entry into the M phase. At that point, further treatment with hyperthermia might induce a total apoptosis. Thus, in this experiment, Chinese hamster ovary (CHO) and MTGB (a murine mammary adenocarcinoma) cells will be treated with steviol alone, steviol with hyperthermia, and hyperthermia alone to evaluate their cytotoxic and apoptotic potential. Their cytotoxicity will be compared to determine if hyperthermia could work with steviol to increase its cytotoxicity and apoptotic effect..

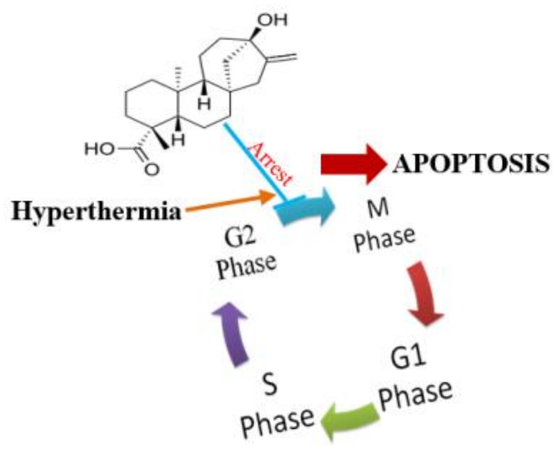


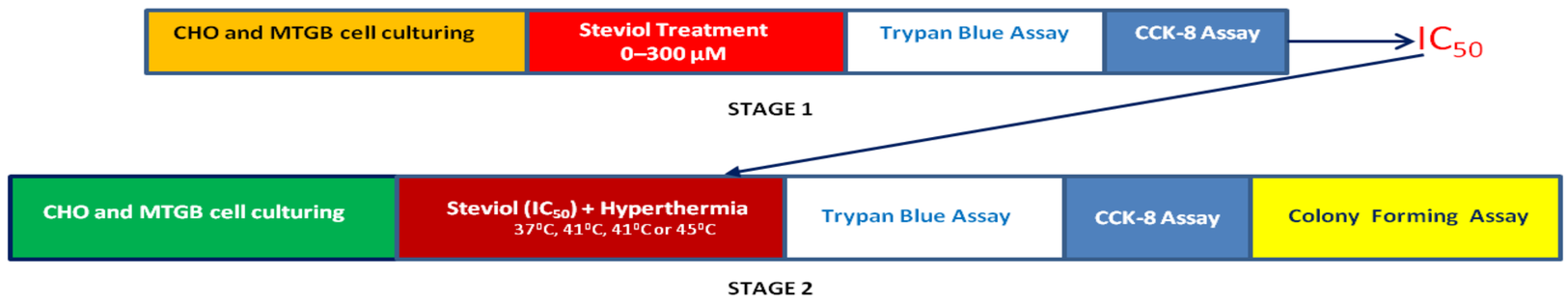
Fig. 2: Proposed interaction between steviol and hyperthermia.

Experimental Protocol:

Stage 1: The purpose of Stage 1 is to determine the dose of steviol at which 50% of the cells will die (i.e. IC50 value). This will be used to set the dose value in Stage 2. The trypan blue assay and CCK-8 assay will be used to determine cell death. CHO and MTGB cells will be used.

Stage 2: Stage 2 will combine hyperthermia and the dose determined by Stage 1. CHO and MTGB cells will be used. Cells will be held at either 37°C, 41°C, 43°C or 45°C for 1 hr using a water bath. Cytotoxicity will be evaluated through the CCK-8 assay, the trypan blue assay, and colony forming assay.

Fig 3: Experimental Plan



Trypan Blue Assay: This assay is use to determine the cell viability and the cell viability is calculated as the number of viable cells divided by the total number of cells within the grids on the hemocytometer. The cells are considered non-viable if they absorb trypan blue.[14]

Cell counting kit- 8 (CCK-8) Assay: CCK- 8 uses a highly water-soluble tetrazolium salt WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium,monosodium salt]. The WST-8 is reduced by dehydrogenases in cells resulting in a yellow colored product (formazan), which is soluble in the tissue culture medium. The amount of the formazan dye generated in cells is directly proportional to the number of living cells.

The Colony Forming Assay allows an evaluation of the differences in reproductive viability (capacity of cells to produce progeny; i.e. a single cell to form a colony of 50 or more cells) between treated and untreated cells.[15]

Significance and Future Work:

Tumor cells have the characteristics of resisting apoptosis and the advancement of cancer cells to resist drug is one of the major reason for unproductive cancer treatment. Thus there is a need for an immediate new therapeutic therapy that can destroy tumor cell with less toxicity or damage to normal cells in the biological system. If steviol shows great efficacy with hyperthermia, it may one day be paired with hyperthermia platforms such as microwave, ultrasound, and nanoparticles which can both be used to induce local hyperthermia and carry chemotherapeutics.

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